IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Michel PAIRET, et al. Examiner: Barbara Badio

Serial No.: 10/776,757 Group Art Unit: 1612

Filed: February 11, 2004 Confirmation No.: 3466

Title: PHARMACEUTICAL COMPOSITIONS BASED ON ANTICHOLINERGICS AND CORTICOSTEROIDS

DECLARATION UNDER 37 C.F.R. §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Thierry Bouyssou, being duly warned, declare that:

I am a citizen of France, residing in Germany.

I have studied Biochemistry at the University of Toulouse (University Paul Sabatier), France from 1979 to 1983 and obtained a Master in Biochemistry degree.

I did my doctoral thesis in Toulouse (Veterinary school) from 1984 to 1987 and received a PhD degree from the Polytechnic Institute of Toulouse, France in 1987.

Since 2001, I have been employed by Boehringer Ingelheim, presently in the Department of Pulmonary Research of Boehringer Ingelheim Pharma GmbH & Co. KG, Germany.

I am not a co-inventor of the above-captioned patent application but I am familiar with the above-captioned patent application (hereinafter referred as the "Pairet application"). I am also familiar with the U.S.P.T.O. Office action dated March 25, 2009, in the Pairet application and

the references that were cited against the claims in the action: Nishimura (article in Allergology International 1999); Banholzer (U.S. Patent No. 5,610,163); and Keller (WO Pub. No. 00/28979).

The following experiments were conducted by me or under my supervision in Germany to show the effects of tiotropium bromide, ciclesonide and their combination in a bronchoprotection model and, thereby, show the unexpected advantage of the invention claimed in the Pairet application:

ANIMALS

Male and female Beagle dogs with a body weight ranging from 10 – 14 kg were obtained from the in-house breeding station. The animals were kept in the kennel of the local animal house, fasted overnight before the experiment, but had free access to drinking water.

ANAESTHESIA AND PREPARATION

Anaesthesia was induced by intravenous bolus injection of 10 mg/kg propofol followed by an intravenous infusion of 30 mg/kg/h propofol into the cephalic vein. The dogs were intubated and then ventilated with volume-controlled pressure with a mixture of room air and oxygen (3:1) using a Siemens respirator at a rate of 15 strokes per minute. Optimal ventilation was assured by regular measurement of acid-base status and oxygen saturation in the blood.
The contralateral cephalic vein was prepared for acetylcholine (ACh) injections. Adhesive electrodes were placed onto the locally shaved skin of the four legs and used for ECG recording the limb leads for evaluation of heart rate.
Transpulmonary pressure was monitored by means of a differential pressure transducer (Sensortechnics GmbH, Puchheim, Germany) using a catheter connected with the endotracheal tube. A Fleisch-tube type 1 (Hugo Sachs Elektronik- Harvard Apparatus GmbH, March Hugstetten, Germany) was connected to the endotracheal tube for pneumotachographic measurement of respiratory flow.
Body temperature was maintained at 37-38°C by means of a heating bed.

EXPERIMENTAL PROTOCOL

A control period of 30 min followed the completion of the instrumentation. Thereafter, a first inhalative treatment was performed followed by an observation interval of 3 hours.

Acetylcholine was repeatedly injected at a dose of 10 µg/kg for the induction of transient bronchospasm (approx. 30 % increase in bronchial resistance) at times: –45, -30, -15, 5, 10, 30, 60, 90, 120, 150 and 180 minutes after the initial treatment. Cardiovascular parameters were evaluated at the same times immediately before induction of bronchospasm.
Bronchoprotection effect provided by the compound was also assessed over 24 hours. Dogs were allowed to wake up after the 3 hour study and were anaesthetized 20 hours afterwards (i.e. 23 hours after drug administration) for measurement of the 24 hour value (as described above).
At the end of the experiment, dogs wake up and are available for further testing after a washout period of at least 4 weeks.

DRUGS

The test compounds were dissolved in 100 % ethanol at concentrations permitting their administration in 5 actuations of 10µl (each actuation) with the use of a soft mist inhaler. Compounds given as combination were also dissolved in the same solution of 100 % ethanol and administered in 5 actuations of 10 µl.
Acetylcholine (Acetylcholine ophthalmicum Dispers) is from Dispersa GmbH (Germering, Germany). Propofol (Propofol-Lipuro 2 %) is from B Braun Melsungen AG, (D-34209 Melsungen, Germany).
Tiotropium bromide and ciclesonide were synthesized by the Department of Chemical Development of Boehringer Ingelheim Pharma GmbH & Co KG.

RESULTS

The time-course of the inhibitory effect of tiotropium bromide in combination with ciclesonide on acetylcholine-induced bronchoconstriction over 24 hours after single administration was investigated in comparison to the respective mono-therapies.

Dogs received ciclesonide (0.1 mg/kg or 0.3 mg/kg) or tiotropium bromide (0.06 µg/kg or 0.1 µg/kg) or combinations of both drugs by inhalation. The following parameters were measured: transpulmonary pressure, pulmonary resistance and heart rate. Measurements were made at different time points (–45, -30, -15, 5, 10, 30, 60, 90, 120, 150 and 180 minutes) after the initial treatment, in the same animal under anesthesia using propofol.
Bronchoprotection of the compound was also assessed 24 hours after drug administration.

Dogs were allowed to wake up after the 3 hour study and were anaesthetized 20 hours afterwards (i.e. 23 hours after drug administration) for measurement of the 24 hour value. At each time point, bronchoconstriction was induced by an intravenous injection of 10 µg/kg acetylcholine (= ACh-challenge). Additionally heart rate was measured.

Ciclesonide applied at 0.1 mg/kg only induced slight bronchoprotection of 5 ± 10 %, 3 hours after drug inhalation which remained constant over 24 hours. The dose of 0.3 mg/kg also displayed weak bronchoprotection of 6 ± 2 % at 3 hours which slightly increased over the time with maximal value of 16 ± 14 % at 24 hours.

Tiotropium bromide displayed a dose-dependent bronchoprotection which reached 35 ± 25 % at 0.06 µg/kg and 57 ± 21 % at 0.1 µg/kg, 3 hours after inhalation. The compound maintined at the end of the 24 hour study period a bronchoprotection of 12 ± 7 % and 37 ± 16 % for the respective doses.

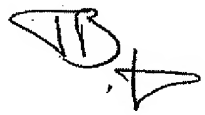
Compared to the efficacy of each mono-therapy, the combination of submaximal doses of ciclesonide (0.1 mg/kg) and tiotropium bromide (0.06 µg/kg) resulted in an unexpected super-additive bronchoprotection of 49 ± 7 % at 3 hours and of 41 ± 14 % after 24 hours.

The combined administration of tiotropium bromide and ciclesonide resulted in a clearly synergistic bronchoprotection in this model. In particular at the lower doses of tiotropium bromide (0.06 µg/kg) in combination with ciclesonide (0.1 mg/kg) this effect appears to be significantly higher than the summarized values of the respective mono-therapies. This is quite apparent at 3 and 24 hours after inhalation of the test compounds.

The results of the mono-therapy of tiotropium bromide (0.06 µg/kg) and ciclesonide (0.1 mg/kg) and their combination together with a control are summarized in the attached graph.

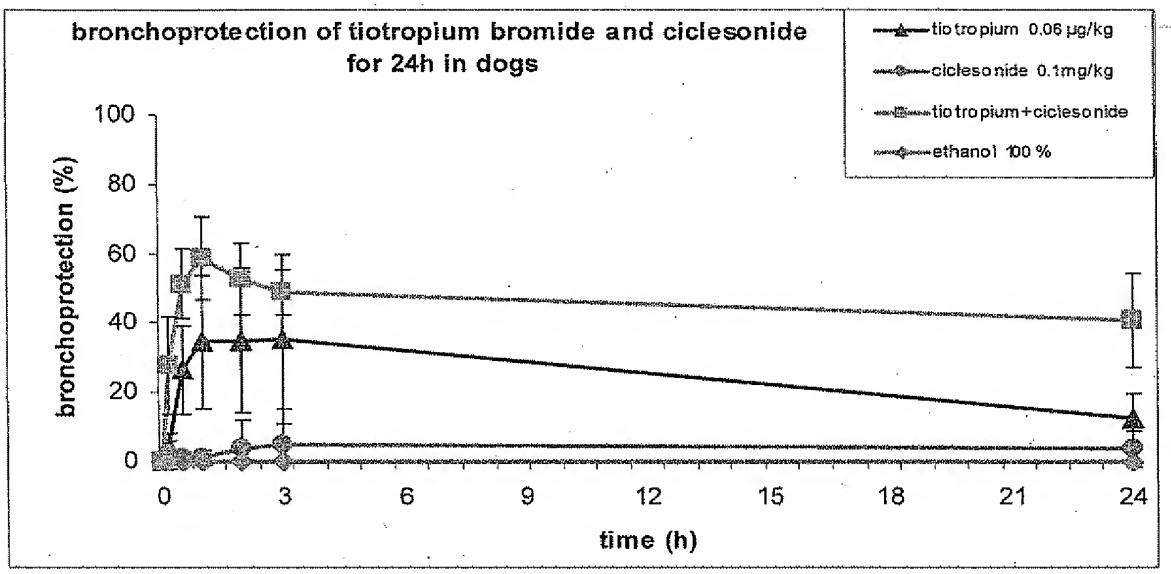
I conclude that the synergistic bronchoprotection properties of the combined administration of tiotropium bromide and ciclesonide would not have been expected by those of ordinary skill in the art from the consideration of the Nishimura (article in Allergology International 1999); Banholzer (U.S. Patent No. 5,610,163); and Keller (WO Pub. No. 00/28979) references.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.



Date:___November/02/2009__

Thierry Bouyssou


bronchoprotection of tiotropium bromide and ciclesonide for 24h in dogs
tiotropium 0.06 µg/kg
ciclesonide 0.1mg/kg
tiotropium+ciclesonide
ethanol 100 %
bronchoprotection (%)
100
80
60
40
20
0
0
3
6
9
12
15
18
21
24
time (h)